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Metabolite Fingerprinting of "The Walking Mango Tree" Bark Extracts by HPLC- UV/DAD, GC-MS, MALDI-TOF Mass Spectrometry

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ABSTRACT

Mangifera indica is being used in indigenous medical systems for the treatment of various diseases. All parts of the plant like stem bark, leaves, flower, fruit pulp and seeds are traditionally used for many diseases. Metabolite profiling in medicinally important plants is critical to resolve the problems associated with standardization and quality control. Metabolite profiling of the bark was performed by HPLC-UV/DAD, GC-MS and MALDI-TOF mass spectrometry. These hyphenated techniques helped in the identification of chemically-diverse metabolites. These include anthocyanins, anthocyanidins, sugars, phenolics and volatile compounds. Five

extracts of bark were prepared utilizing Aqueous, Acetone, Chloroform, Methanol & Petroleum Ether. The present work was undertaken to analyze compounds present in the walking mango tree bark extracts (WMTBE) and compared with the normal mango tree bark extracts (NMTBE). HPLC- UV/DAD, MALDI-TOF & GC-MS were utilized for screening of the compounds present in the extract.

Keywords: HPLC- UV/DAD, MALDI-TOF, GC-MS, Screening, Profiling

Abbreviations: WMTBE- Walking mango tree bark extract, NMTBE- Normal mango tree bark extract, MTT- [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide], HPLC- High performance liquid chromatography, MALDI- Matrix assisted laser desorption/ionization time of flight, GC MS- Gas chromatography mass spectroscopy.

1. INTRODUCTION

The search for newer and effective antimicrobials is a continuous scientific exercise due to the resistance of microorganisms (bacteria, fungi, parasites, viruses) to currently existing drugs (El-Aneed, A., 2009). Plants undoubtedly have been a veritable source of medicine to mankind. In recent years, in the search for new drugs and escalating public demand, researchers have turned to plant sources for the active molecules (Ajila, C. M *et al.* 2007). Plants continue to be a major source of commercially consumed drugs. Even many synthetic drugs have their origin from natural plant products (Barreto, J. C. *et al.* 2008). The trend of using natural products has increased in recent years and the active plant extracts are frequently screened for new drug discoveries.

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens (Berardini, N., 2004). Mango stem-bark aqueous extract has been reported to possess anti-inflammatory, analgesic and immune-protective effects. A number of biological activities of mangiferin have been suggested, including anti-diabetic and anti-inflammatory abilities (Dorta, E. *et al.*2011 & 2012).

Mangifera indica L. (Anacardiaceae) grows in the tropical and subtropical regions and its parts are used commonly in folk medicine for a wide variety of conditions. *Mangifera indica* L. (Anacardiaceae) is one of the most important tropical plants marketed in the world (Engels C, 2009 & 2010). There are many traditional medicinal uses for the bark, roots and leaves of *M. indica* throughout the globe. *Mangifera indica* is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains, malaria and diabetes (Landete, J. M., 2011). A fluid extract of bark is used to cure monorrhagia, leucorrhoea, bleeding piles and incase of haemorrage from nose (Ornelas-Paz Jde J., 2007).

The potential of plants as a source for new drug and botanical pesticides is still largely unexplored. This is also true in India and only a small percentage of plants of this region have been evaluated (Rakholiya Kalpna, 2012). This led the author to screen the extracts of bark of WMT and NMT. By this study, we could improve our understanding of the present in the extracts and also to detect the unknown molecules which are absent in NMT, but present in WMT.

2. MATERIALS & METHODOLOGIES

2.1 Samples:

Fresh bark samples were collected from the walking mango tree, Gujarat as well as from normal mango tree from Bengaluru. Sample were air dried at room temperature and crushed into small particles using mortar& pestle; stored in a Ziploc bags for further use.

2.2 Extraction Methodology:

Soxhlets apparatus was utilized for extraction of various pigments & molecules from the bark powder; rotaevaporator was used for removing the solvent to determine the amount weight of compounds that got dissolved in the solvents. The determined samples are stored at 4°C for further use.

2.3 Solvents:

Acetone (least polar)
Aqueous (high polar)
Chloroform (lipophilic)
Methanol (high polar)
Petroleum Ether (non-polar)

2.4 Instruments used:

Soxlets apparatus
Rota-evaporator: Heidolph

Heating mantle: IKA® C-MAG HS 7
Recirculating cooler: Julabo F25
Flat shaker: IKA® KS 260 basic
BIO RAD: Model 680; microfigure reader
HPLC- UV/DAD
MALDI- TOF
GC-MS

3. RESULTS

The analyzed data/ spectra's have been represented below for better understanding of the compounds.

Figure 1 HPLC Spectra - WMT Aqueous Extract (270-380 nm)

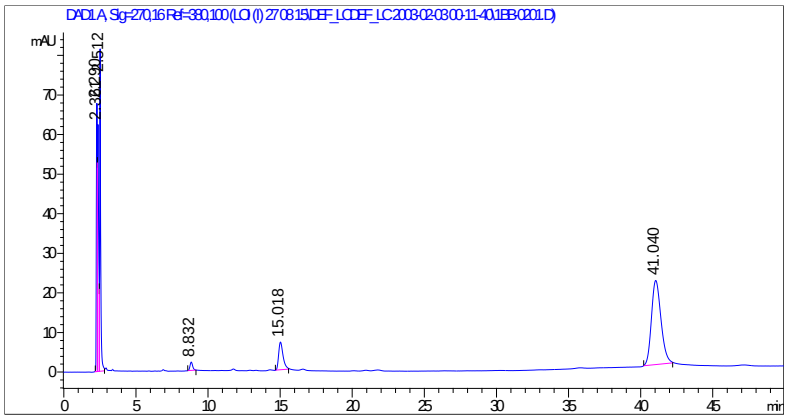

Table 1 HPLC- WMT Aqueous Extract (270-380nm)

No.	Time	Area	Height	Width	Area%	Symmetry
1	2.29	332.3	67.8	0.0772	15.175	1.266
2	2.361	334.7	62.4	0.0746	15.286	0.439
3	2.512	367.4	82.2	0.0641	16.782	0.602
4	8.832	28.1	2.1	0.2037	1.283	0.809
5	15.018	149	7	0.3198	6.805	0.66
6	41.04	978.1	21.3	0.7083	44.67	0.779

Figure 2 HPLC Spectra- NMT Aqueous Extract (270- 380 nm)

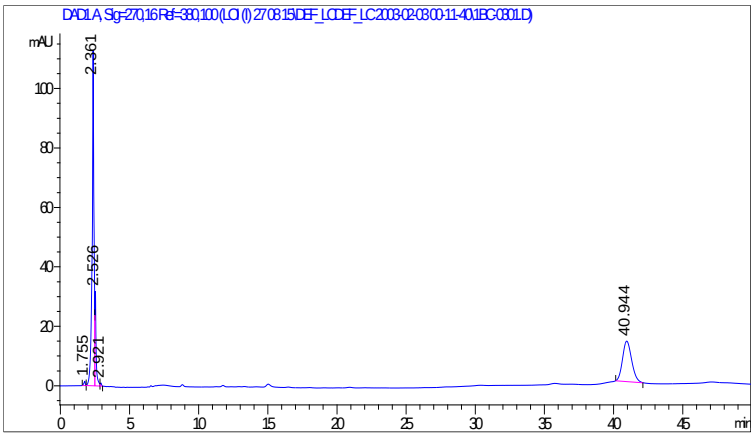

Table 2 HPLC- NMT Aqueous Extract (270-380nm)

No.	Time	Area	Height	Width	Area%	Symmetry
1	1.755	11.4	1.5	0.1073	0.616	1.665
2	2.361	1032.1	112.9	0.1247	55.784	1.186
3	2.526	172	31.9	0.075	9.294	0.403
4	2.921	7	1.1	0.0929	0.376	0.846
5	40.944	627.7	13.7	0.6924	33.929	0.809

Figure 3 MALDI- TOF of WMT & NMT overlaps spectrums

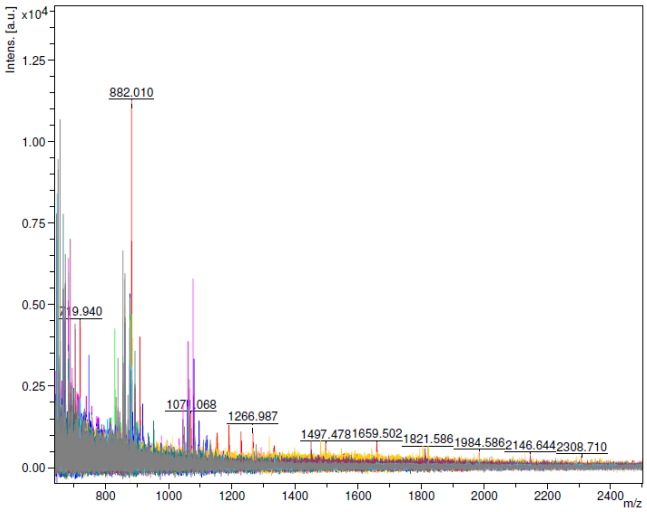

Figure 4 MALDI- TOF of WMT & NMT spectrums

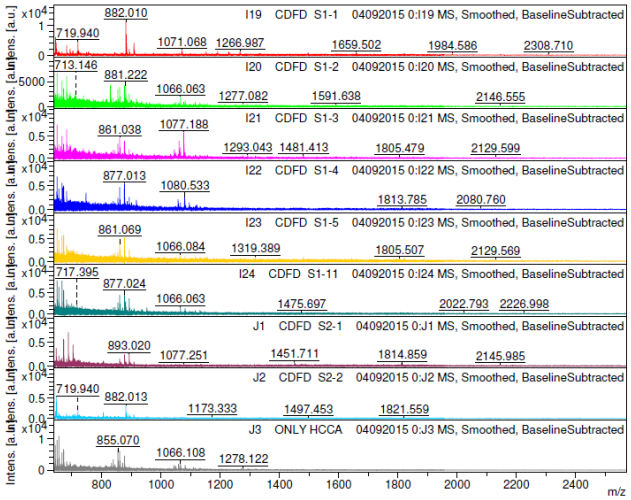

Figure 5 GC MS Spectra- Fatty Acids- WMT

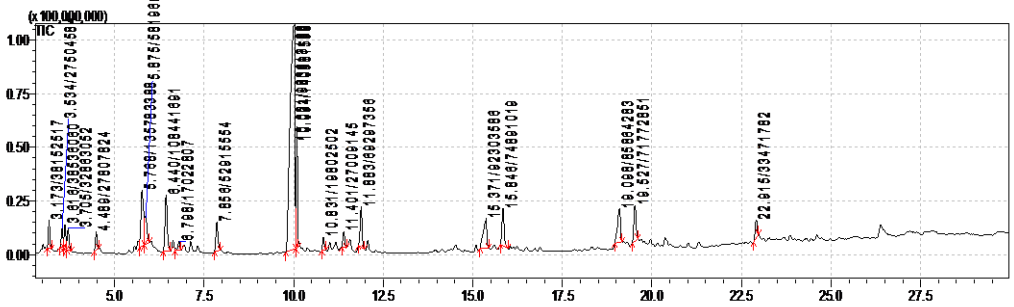

Table 3 GC MS Spectra- Fatty Acids- WMT compounds

N° Pic	NAME OF THE COMPOUND	M/Z	RT	AREA	SI
1	Octane, 6-ethyl-2-methyl-	71	3.005	3325161	95
2	Nonane, 4,5-dimethyl-	71	3.005	3325161	91
3	5,8-Dimethylenebicyclo[2.2.2]oct-2-ene	175	3.172	17415925	96
4	1-Nonene, 4,6,8-trimethyl-	69	3.616	8014886	98
5	Heptafluorobutanoic acid, nonyl ester	55	4.489	5695342	99
6	Decane, 3,7-dimethyl-	71	5.406	1356434	95
7	1-Undecene	55	5.769	28732313	98
8	1,2-Bis(trimethylsilyl)benzene	191	6.439	36172651	98
9	1-Undecene, 7-methyl-	69	6.631	3887780	95
10	2-Isopropyl-5-methyl-1-heptanol	69	6.799	3576231	96
11	Methyl 4,6-decadienyl ether	55	7.857	7607850	99
12	Pentafluoropropionic acid, undecyl ester	55	9.933	1.05E+08	94
13	Propanoic acid, octyl ester	57	10.094	24669027	97
14	1-Decanol, 2-methyl-	69	10.831	4132485	97
15	Cyclopentane, 1-pentyl-2-propyl-	69	11.401	8184900	97
16	3-Tridecene, (E)-	55	11.884	9658738	99
17	Ethanol, 2-(octyloxy)-	69	12.07	3220615	96
18	2-Trifluoroacetoxydodecane	57	12.293	486956	93
19	Tetradecane	71	14.141	1572812	91
20	Butanoic acid, 2-methyl-, octyl ester	74	14.528	1666075	83
21	Methoxyacetic acid, 6-ethyl-3-octyl ester	71	14.778	516001	92
22	2-Methyl-Z-4-tetradecene	73	15.371	12502317	95
23	2-Heptafluorobutyroxytetradecane	57	15.844	9779038	99
24	Isotridecanol-	69	16.229	1084003	93
25	1-Methoxy-3-(2-trimethylsilyloxyethyl)nonane	73	16.874	1211215	75
26	Dodecanoic acid	73	19.099	14118348	94
27	2-Heptafluorobutyroxypentadecane	69	19.096	6559826	84

28	1-Hexadecene	57	19.526	8752888	97
29	1,19-Eicosadiene	57	29.448	51714	92

Figure 6 GC MS Spectra- Fatty Acids- NMT

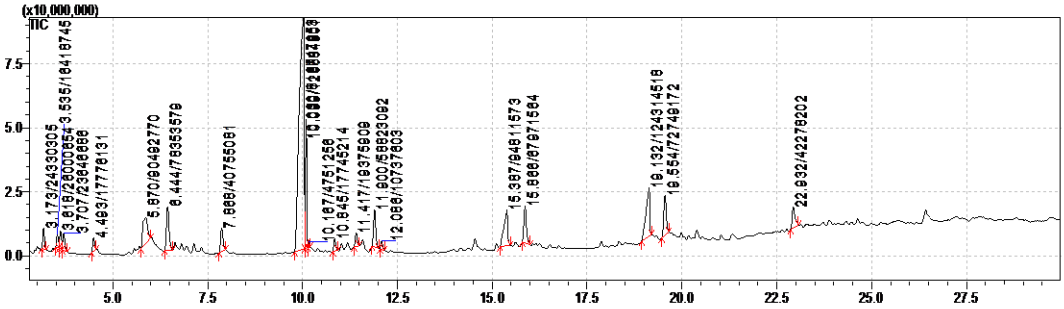

Table 4 GC MS Spectra- Fatty Acids- NMT compounds

N° Pic	NAME OF THE COMPOUND	M/Z	RT	AREA	SI
1	Octane, 6-ethyl-2-methyl-	71	3.005	2539587	94
2	Nonane, 4,5-dimethyl-	71	2.953	1104943	88
3	5,8-Dimethylenebicyclo[2.2.2]oct-2-ene	175	3.172	12096763	94
4	Benzene, (epoxyethyl)-, (R)-	91	3.42	3658351	83
5	1-Nonene, 4,6,8-trimethyl-	69	3.535	5103719	96
6	Heptafluorobutanoic acid, nonyl ester	55	4.493	4227901	99
7	1-Hexanol, 5-methyl-2-(1-methylethyl)	57	4.287	52686	81
8	Decane, 3,7-dimethyl-	71	5.413	870601	96
9	1-Undecene	55	5.816	20691288	94
10	1,2-Bis(trimethylsilyl)benzene	191	6.443	28373201	97
11	1-Undecene, 7-methyl-	69	6.643	3186054	93
12	1-Undecene, 7-methyl-	69	6.808	2600791	94
13	2-Isopropyl-5-methyl-1-heptanol	69	6.949	2129601	96
14	Methyl 4,6-decadienyl ether	55	7.869	6456078	99
15	3-Heptafluorobutyroxydodecane	55	9.943	83610337	74
16	Propanoic acid, octyl ester	57	10.099	21455890	97
17	1-Decanol, 2-methyl-	69	10.845	3444293	96
18	Cyclopentane, 1-pentyl-2-propyl-	69	11.417	6049354	97
19	3-Tridecene, (E)-	55	11.9	8463376	99
20	2-Trifluoroacetoxydodecane	69	12.087	2823946	94
21	Undecanoic acid, 10-methyl-, methyl ester	74	14.548	3479486	89
22	2-Methyl-Z-4-tetradecene	73	15.386	13208666	92
23	2-Heptafluorobutyroxytetradecane	55	15.865	8980508	98
24	Dodecanoic acid	57	19.133	13394861	94
25	1-Hexadecene	57	19.551	9640021	97
26	3-Hexadecene, (Z)-	69	19.55	5084849	90
27	1-Heptadecene	57	22.932	4570898	93

Figure 7 GC MS Spectra - Organic acids- WMT

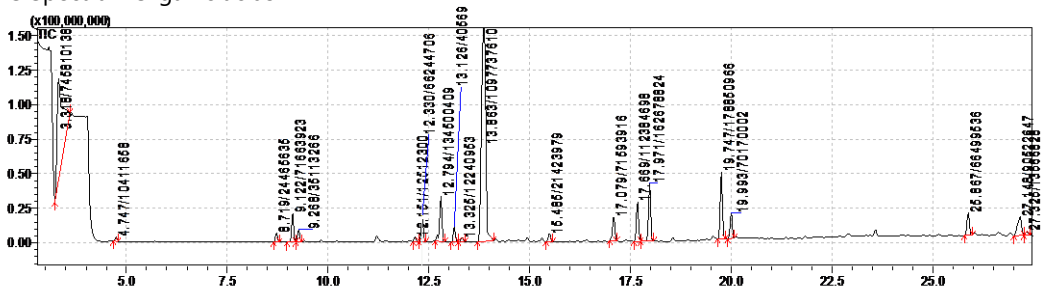

Table 5 GC MS Spectra- Organic acids- WMT compounds

N° Pic	NAME OF THE COMPOUND	M/Z	RT	AREA	SI
1	1,2-Dioxetane, 3,4,4-trimethyl-3-[[(trimethylsilyl)oxy]methyl]	72	3.488	1.17E+08	79
2	Silane, trimethyl[2-methylene-1-(4-pentenyl)cyclopropyl]	73	4.75	6352396	94
3	1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)	299	8.712	6919136	91
4	Butanedioic acid, 2,3-dimethyl-, bis(trimethylsilyl) ester	147	9.12	18283826	99
5	2,6-Octadiene, 1,8-bis(trimethylsilyl)	73	9.269	18589552	99
6	1-Tridecene	55	11.21	3170441	98
7	Pentanoic acid, 2-propyl-, tert-butyldimethylsilyl ester	243	12.33	14468548	96
8	3-Methyl-hexahydro-pyrano[3,2-b]pyran-2-one	71	12.79	31640998	97
9	Silane, trimethyl[[p-(trimethylsiloxy)benzyl]oxy]	267	13.12	6566207	95
10	Decanoic acid, trimethylsilyl ester	73	13.32	2658508	95
11	Methyl 6,8-dodecadienyl ether	55	13.84	75399575	96
12	1-Tetradecene	55	13.84	75140093	93
13	Undecanoic acid, 11-fluoro-, trimethylsilyl ester	73	15.49	3623689	96
14	Bis(heptamethylcyclotetrasiloxy)siloxane	73	17.09	21282165	94
15	Propanoic acid, 3-mercapto-, 2-ethylhexyl ester	57	17.67	14362786	99
16	Silane, trimethyl(1-methyldodecyloxy)	73	17.97	20879125	99
17	Dodecanoic acid, trimethylsilyl ester	73	19.75	21838471	99
18	13,13-Dimethyl-3,6,9-trioxa-13-silatetradecan-1-ol	163	19.99	15117256	93
19	3-(1,5-Dimethyl-hexa-1,4-dienyl)-2,2-dimethyl-4-trimethylsilylcyclopentanol	73	25.87	9485983	96
20	1,11-Undecanedioic acid, di(trimethylsilyl) ester	73	27.16	14356499	94

Figure 8 GC MS Spectra - Organic acids- NMT

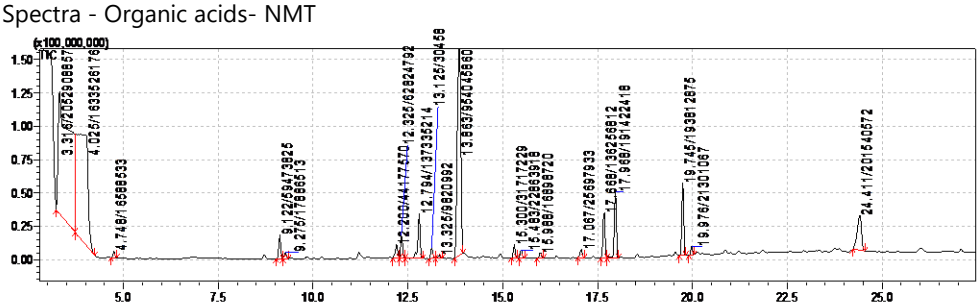

Table 6 GC MS Spectra- Organic acids- NMT compounds

N° Pic	NAME OF THE COMPOUND	M/Z	RT	AREA	SI
1	Bis(trimethylsilyl)alanine, methyl ester	188	3.336	62504708	84
2	Silane, trimethyl[2-methylene-1-(4-pentenyl)cyclopropyl]	73	4.751	9455126	92
3	1-Ethyl-2-pentamethyldisilanyloxycyclohexane	299	8.707	3613060	92
4	Butanedioic acid, 2,3-dimethyl-, bis(trimethylsilyl) ester	147	9.12	17423983	97
5	2,6-Octadiene, 1,8-bis(trimethylsilyl)	73	9.271	9869618	98
6	Benzoic acid, 2-methyl-, trimethylsilyl ester	119	9.844	1254249	91
7	1-Tridecene	55	11.209	3276651	97
8	Silane, [[3,3-dimethyl-4-methylene-2-(trimethylsilyl)-1-cyclopenten-1-yl]methoxy]trimethyl	73	12.206	11427950	96
9	Pentanoic acid, 2-propyl-, tert-butyldimethylsilyl ester	243	12.327	14073543	96
10	3-Methyl-hexahydro-pyrano[3,2-b]pyran-2-one	71	12.788	31111255	97
11	Silane, trimethyl[[p-(trimethylsiloxy)benzyl]oxy]	267	13.124	5209555	97
12	Decanoic acid, trimethylsilyl ester	73	13.324	1869874	94
13	Methyl 6,8-dodecadienyl ether	73	13.846	65495237	98
14	Mono-TMS of (pyridoxine-H2O)	73	15.303	7721985	97
15	Undecanoic acid, 11-fluoro-, trimethylsilyl ester	73	15.487	3762672	97
16	3-Dodecanol, 3,7,11-trimethyl	73	15.996	3358497	93
17	Bis(heptamethylcyclotetrasiloxy)siloxane	73	17.073	7324945	97
18	Isooctyl 3-mercaptopropionate	57	17.669	18675955	99
19	7-Oxooctanoic acid, 2-trimethylsilylethyl ester	73	17.967	21687712	99
20	Dodecanoic acid, trimethylsilyl ester	73	19.744	19833145	98
21	4-Thiazolidinecarboxylic acid, 2-thioxo	163	19.975	5770826	91
22	Threitol, 2-O-heptyl-	57	24.42	57398261	91

4. DISCUSSION

In this study we have utilized only aqueous extract for all the analysis. Since the localities soak the bark in the water for overnight & later consume it for their ailments. Hence our intension was to understand those compounds which are soluble in aqueous solvent; which could be a better medicine for healing purposes. Though I do agree that not all the organic compounds are soluble in aqueous solvent. The overall purpose was to find the difference in the compounds among different solvents. Moreover aqueous solvent doesn't cause any side effects; unlike those of organic solvents.

As per HPLC-UV/DAD spectra, we can observe at RT 9 & 15 unique in WMT, which is not as strong in NMT. So these compounds are the targeted ones, which can be screened for further studies. Similarly when the aqueous extract of WMT & NMT emitted in MALDI, we have observed that the compounds are organic compounds & not proteins. This question was aroused while performing apoptosis studies. Hence we wanted to find out, if the apoptosis was caused due to the protein or any organic compound. When screened through MALDI, we confirmed that proteins have no role in apoptosis. The reason is quite simple. In the extraction process via Soxhlets, the protein gets denatured due to excessive heat. But there might be a chance; if a protein survived the heat. So for confirming its actions, MALDI-TOF was performed. If we observe any spectra below 10 KDa, it resembles organic compound. If above 10KDa, it is protein. Since all our MALDI-TOF spectra were below 10KDa, we confirmed that the apoptosis was due to organic compound & not by any protein. So our next step was to isolate every single compound present in the extract. So GC- MS was performed.

In GC-MS spectra, we have performed only for organic acids & fatty acids. For fatty acids we have observed 29 compounds for WMT & 27 compounds for NMT. Similarly for organic compounds we have 20 compounds for WMT & 22 compounds for NMT. Now the actual quest arises. Since we have a huge list of compounds, we need to isolate and collect every single compound and this has to be check upon cell lines for apoptosis. Once we find out the compound, we should carry upon animal studies (clinical trials). Since this will cost more than expected; an alternative way to understand this process would be via docking the molecule to the targeted protein. By docking studies we can understand if our isolated compound can interact with the protein or not. If the compound gets bounded, then we might have a chance to cure the disease. It is not as easy as we write. Combination of molecules needed to be docked onto a protein for better results. Once we find out the suitable molecule, we can synthesis this in & can be used for clinical studies.

5. CONCLUSION

In this preceding we have proceeded with the spectroscopy techniques in analyzing the compounds present in the WMTBE & NMTBE. Only a few compounds in were observed in HPLC spectra, but this was compromised with GC MS spectra, which exhibited all the compounds present in these extracts. MALDI- TOF was used to determine if the compound was a protein or organic molecule. Hence with this data we can proceed ahead with docking studies.

FUTURE ISSUES

Also keeping in mind that we only have one tree available, resources has to utilized very carefully, without causing more harm to the tree. GC MS for carbohydrates has to perform for better understanding & utilization of other organic solvents for isolation of unidentified compounds. Docking studies need to be performed for better understandings of the compound interactions with the protein.

DISCLOSURE STATEMENT

This work was supported through INSA-NASI-INSc fellowship, under the supervision of Dr.SK Manna, CDFD, HYD, IND.

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